Disclosure Statement, with the Examiner's initials in the left column indicating that each of the references have been considered, be returned to the undersigned.

Regarding the Amendments

Claims 38, 41, 42, 45, 46 and 54 have been amended in order to address an informality. In particular, the amendments were made in order to conform the claims with the elected subject matter. Thus, the amendments do not add new matter and entry thereof is respectfully requested.

Regarding the Sequence Listing

Submitted herewith are Substitute paper and computer readable copies of the Sequence Listing in accordance with 37 C.F.R. §1.821-1.824. Submitted concurrently herewith is an executed statement under 37 C.F.R. §1.821(f) and (g) that the paper and computer readable copies are identical and that no new matter has been added. Accordingly, entry of the Substitute Sequence Listing is respectfully requested.

REJECTIONS UNDER 35 U.S.C. §112

The rejection of claims 43 and 44 under 35 U.S.C. §112, first paragraph, as allegedly lacking an adequate written description, is respectfully traversed. The Examiner indicates that "the nucleotide sequences that encode *all* variants or functional subsequences thereof of gut endocrine promoters, encompassed within the genus of nucleotide molecules of gut endocrine promoters have not been disclosed." [paragraph bridging pages 4 and 5; Emphasis added] The Examiner acknowledges that the "specification discloses various gut endocrine promoters on pages 14-15 but does not disclose any variants or functional subsequences of gut endocrine promoters embraced by the claims."

The specification adequately describes claims 43 and 44. Applicants first point out that contrary to the statement in the Office Action, the specification need not disclose "the nucleotide sequences that encode all variants or functional subsequences thereof of gut endocrine promoters, encompassed within the genus of nucleotide molecules of gut endocrine promoters" in order to satisfy the written description requirement under 35 U.S.C. §112, first paragraph. [Office Action, paragraph bridging pages 3 and 4] Rather, to satisfy the written description requirement the specification need only apprise the skilled artisan of the invention in sufficient

detail to demonstrate Applicants had possession of the invention. Possession may be shown by "any description of sufficient, relevant, identifying characteristics so long as a person skilled in the art would recognize that the inventor had possession of the claimed invention." *Purdue Pharma L.P. v. Faulding Inc.*, 230 F.3d 1320, 1323 (Fed. Cir. 2000).

In the present case, those skilled in the art were apprised of the sequences of many different gut endocrine promoters and enhancers, and the elements therein that confer promoter function, at the time of the invention. Thus, given that those skilled in the art would have known the sequences important for promoter function, they also would have known which sequences could be mutated or deleted without destroying promoter function and, consequently, functional promoter variants and subsequences. As such, those skilled in the art would be apprised of sufficient relevant, identifying characteristics of functional gut endocrine promoter and enhancer variants and subsequences thereof.

For example, as to the GIP promoter, the specification discloses the sequence and location of several transcriptional control elements including two TATA boxes and two CCAAT-like boxes. The specification further discloses that there are potential AP-1 and AP-2 sites, a cAMP response element, a potential insulin-response element located upstream of the putative transcription start site and two GATA binding motifs (page 13, lines 10-16). The specification further discloses that mutations in the distal and proximal GAT motifs reduced GIP promoter activity 90% and 35%, respectively (page 13, lines 17-19). The specification also discloses that a GIP promoter containing one or more of these variants or subsequences is an example of a sequence that can retain glucose-regulatable or tissue specific (gut) expression of an operably linked nucleic acid. (page 13, lines 21-26). Thus, the specification discloses which sequences are important for GIP promoter function and, as such, the sequences that could be varied or deleted and yet still confer GIP promoter function.

In addition to the teachings in the specification, other investigators have characterized GIP variants and subsequences having activity. For example, 5' deletion mutants of the human GIP promoter activity (a 1256bp AvaII proximal promoter fragment linked to a chloramphenicol acetyltransferase) in a Syrian hamster β-cell line revealed that the sequence region between –180 and +14 conferred basal promoter activity in the insulinoma cell line (Someya et al., 1993 FEBS Lett, 317:67-73). Deletion analysis of the rat GIP promoter in STC-1 cells revealed that the first 193bp upstream of the transcription initiation site was able to direct approximately the same

level of expression as a 943bp promoter fragment in STC-1 cells (Boylan et al., 1997 J Biol Chem, 272:17438-17443). Thus, in view of the foregoing, the skilled artisan would have known at the time of the invention that a GIP subsequence from about -180 and +14 or about the first 193bp upstream of the transcription initiation site would have at least some GIP promoter activity.

Furthermore, DNase I footprinting and gel mobility shift assays identified one near-canonical and one atypical cAMP response elements (CRE) at positions –351 (AGACGTGA) and –158 (TCACCAAT), respectively. Mutation of both CRE sites indicates that they are essential for basal promoter activity (Someya et al., 1993 FEBS Lett, 317:67-73). Thus, one skilled in the art would also have known that these CRE's are important for GIP promoter function and, as such, that mutating the CRE elements would likely reduce GIP promoter function.

As to promoters and enhancers for expression in gut other than GIP promoter, Applicants recognize that gut endocrine promoter sequences may be different from each other (see, e.g., Table 1, page 15). However, given the knowledge in the art regarding the elements that are important for their activity, those skilled in the art would know which sequences, if varied or deleted, are likely to reduce function of that promoter or enhancer. Again, as with the GIP promoter, because those skilled in the art would have known which sequences are important for activity of the various gut endocrine promoter and enhancers, those skilled in the art would also know variants and subsequences of gut endocrine promoters and enhancers that would be functional.

Analysis of Secretin deletion mutants in HIT and STC-1 cells revealed that sequences between 174 and 53 bp upstream from the transcriptional start site confer maximal expression (Wheeler et al., 1992 Mol Cell Biol, 12:3531-3539). Four cis-acting elements within this 5' promoter region have been characterized by transient expression assays (Mutoh et al., 2000 Aliment Pharmacol Ther, 14 Suppl 1:170-175; Nishitani et al., 1995 J Clin Gastroenterol, 21 Suppl 1:S50-55). The basic helix-loop-helix protein BETA2 functionally interacted with p300 to activate transcription of the secretin gene (Mutoh et al., 1998 Genes Dev, 12:820-830). BETA2 binds to the E box in the secretin gene. A transgene containing a reporter gene linked to 1.6kb of 5' flanking sequence of the rat secretin gene conferred tissue-specific, developmentally regulated expression in mice (Lopez et al., 1995 J Biol Chem, 270:885-891).

Gastrin transcription has been shown to be increased by epidermal growth factor (Godley and Brand, 1989 Proc Natl Acad Sci U S A, 86:3036-3040). In the human gastrin promoter, an EGF response element (gERE) was located between -54 to -68 bp 5' of the transcription initiation site (Merchant et al., 1991 Mol Cell Biol, 11:2686-2696). Gastrin transcription in islet cells is activated by a cis-regulatory sequence containing a RAP1-like binding site (Simon et al., 1994 FEBS Lett, 351:340-344). The transcription factors binding to this site were later identified to be the Sp transcription factor members Sp1 and Sp3 (Simon et al., 1997 FEBS Lett, 411:383-388). Mutational analysis of the human gastrin promoter further identified a positive cis-regulatory element (CACC) from position -109 to -100 bp (Tillotson et al., 1994 J Biol Chem, 269:2234-2240). RIN ZF, a novel member of the Cys2-His2 zinc finger gene family, exhibited sequence-specific binding to the gastrin CACC element and regulated gastrin gene expression by interfering with Sp1 transactivation (Tillotson et al., 1994 J Biol Chem, 269:2234-2240).

The Cholecystokinin (CCK) gene is composed of three exons interrupted by two introns and maps to the short arm of human chromosome 3 (Deschenes et al., 1985 J Biol Chem, 260:1280-1286; Takahashi et al., 1986 Gene, 50:353-360). The region from –100 to –20 relative to the transcriptional start site contains an E-box element (-97 to –92 CANNTG), a combined CRE/TRE element (-79 to –73), a GC-rich box (-39 to –32) and a TATA-box.

The proglucagon gene is composed of six exons and five introns and spans approximately 10kb (Heinrich et al., 1984 J Biol Chem, 259:14082-14087; White and Saunders, 1986 Nucleic Acids Res, 14:4719-4730). Transfection studies have shown that 300 base pairs of the 5'-flanking region of the rat proglucagon gene permitted specific expression in islet cell lines and this permitted identification of three transcriptional control elements (Philippe et al., 1988 Mol Cell Biol, 8:4877-4888). The G2 (-181 to –202) and G3 (-265 to –286) elements independently displayed enhancer-like functions in alpha cells. The G1 proximal promoter element (-52 to –100) exhibited low intrinsic transcriptional activity but was important for specific expression of the proglucagon gene in alpha cells. A composite DNA control element, G4, localized upstream of G1 between nucleotides -100 and -140 was identified and shown to function as an islet-specific activator in both glucagon- and insulin-producing cells but not in nonislet cells (Cordier-Bussat et al., 1995 Mol Cell Biol, 15:3904-3916). A cyclic-AMP responsive element (CRE at positions -291 to -298) was also identified (Knepel et al., 1990 Mol Cell Biol, 10:6799-6804).

The chromogranin A gene is composed of eight exons encoding a 445 amino acid mature protein (Mouland et al., 1994 J Biol Chem, 269:6918-6926; Wu et al., 1991 J Biol Chem, 266:13130-13134). A glucocorticoid response element located at position -583 to -597 bp confers glucocorticoid regulation of the gene (Rozansky et al., 1994 J Clin Invest, 94:2357-2368). Deletion analysis and scanning mutagenesis of the mouse chromogranin A promoter identified an Sp1/Egr-1 site spanning -88 to -77 base pairs (bp) and a cyclic AMP-responsive element (CRE) at -71 to -64 bp; both sites are important for gastrin-dependent chromogranin A transactivation (Hocker et al., 1998 J Biol Chem, 273:34000-34007). Gastrin stimulation increased cellular Sp1 protein levels and Sp1-binding to the chromogranin A -88 to -77 bp promoter element, as well as binding of CREB to its consensus motif at -71 to -64 bp.

The chromogranin B gene is composed of five exons encoding a 657 amino acid mature protein (Pohl et al., 1990 FEBS Lett, 262:219-224). The promoter region of the chromogranin B gene is GC-rich and contains a CATAA motif, a cAMP-responsive element and an Sp1 binding site (Pohl et al., 1990 FEBS Lett, 262:219-224). 5'-Deletions of the mouse chromogranin B promoter localized neuroendocrine cell type-specific expression to the proximal chromogranin B promoter (from -216 to -91 bp) (Mahapatra et al., 2000 Endocrinology, 141:3668-3678). This region contains an E box (at [-206 bp]CACCTG[-201 bp]), four G/C-rich regions (at [-196 bp]CCCCGC[-191 bp], [-134 bp]CCGCCCGC[-127 bp], [-125 bp]GGCGCCGCC[-117 bp], and [-115 bp]CGGGGC[-110 bp]), and a cAMP response element (CRE; at [-102 bp]TGACGTCA[-95 bp]).

In sum, those skilled in the art would be apprised of functional gut endocrine promoter and enhancer variants and subsequences thereof because many sequence elements within gut endocrine promoters and enhancers important for function were known in the art, as discussed above. Thus, as functional gut endocrine promoters and enhancers having variations or deletions would be recognized by those skilled in the art, claims 43 and 44 are adequately described. Accordingly, Applicants respectfully request that the rejection under 35 U.S.C. §112, first paragraph, as allegedly lacking an adequate written description, be withdrawn.

The rejection of claims 31 to 55 under 35 U.S.C. §112, first paragraph, as allegedly lacking enablement is respectfully traversed. The Examiner indicates allegedly that "the specification fails to provide any relevant teachings or specific guidance or working examples

with regard to the production of insulin *in vivo, by way of the claimed methods*, that results in therapy or prevention of diseases, such as diabetes or obesity." [Emphasis added; Office Action, page 6] Further grounds for rejection relate to difficulties associated with *in vivo* gene therapy, such as "targeting, levels of expression of a therapeutic protein necessary to provide therapy, and mode of administration." [Office Action, page 7]

Claims 31 to 55 are adequately enabled. The proper standard for satisfying the enablement requirement under 35 U.S.C. §112, first paragraph, is whether the specification teaches one skilled in the art how to make and how to use the invention without undue experimentation. In re Wands 858 F.2d 731, 737 (Fed. Cir. 1988). As long as the specification discloses at least one method for making and using the invention that bears a reasonable correlation to the entire scope of the claim the enablement requirement is satisfied. In re Fisher 427 F.2d 833, 839 (CCPA 1970).

Claims 31 to 55 are directed to methods of treating a subject having, or at risk of having, a disorder treatable by producing a therapeutic protein in a mucosal tissue, comprising contacting mucosal tissue cells in the subject transformed with a polynucleotide comprising an expression control element in operable linkage with a nucleic acid encoding the therapeutic protein with a nutrient that induces production of the protein in an amount effective to treat the disorder. Applicants respectfully point out that the claims do not recite transformation of mucosal cells but "contacting mucosal tissue cells in the subject transformed with a polynucleotide comprising an expression control element in operable linkage with a nucleic acid encoding the therapeutic protein with a nutrient that induces production of the protein in an amount effective to treat the disorder." Thus, as claims 31 to 55 recite that the subject has transformed mucosal cells, the invention can be practiced without employing *in vivo* gene therapy. Accordingly, the particular "mode of administration" for transforming mucosal cells into the subject or the mode of introducing transformed mucosal cells into the subject is irrelevant. The ground for rejection relating to gene therapy modes of administration should therefore properly be withdrawn.

As to the alleged absence of a working example for the production of a therapeutic protein *in vivo* resulting in treatment or prevention of disease, the specification exemplifies an *in vivo* working model in which contacting mucosal tissue cells in the animal transformed with a polynucleotide comprising an expression control element in operable linkage with a nucleic acid encoding insulin with a nutrient induced production of insulin in an amount effective to treat the

disorder. In particular, transgenic mice harboring a transformed insulin gene regulated by the GIP promoter were produced in which insulin production in gut was meal (glucose) regulated (Example II, pages 46-47). In control non-transgenic animals, destruction of pancreas β-cells with STZ produced hyperglycemia. In contrast, STZ treated transgenic animals had normal blood glucose levels and rapidly disposed of oral glucose as did normal (non-transgenic non-STZ treated) age matched control animals (Example III, pages 50). The insulin produced was meal-responsive and the amount was sufficient to prevent mice from developing diabetes (page 48, lines 13-21; see, also, Figures 11A and 11B). Thus, contrary to the statement in the Office Action, the specification exemplifies a working *in vivo* animal model in which insulin in mucosal tissue was produced in an amount effective to treat hyperglycemia by contacting the mucosal tissue cells in the animal transformed with a polynucleotide comprising an expression control element in operable linkage with a nucleic acid encoding insulin with a nutrient, as is claimed.

To further corroborate that the invention is adequately enabled as claimed, submitted herewith as Exhibit A is a sworn Declaration under 37 C.F.R. §1.132 and accompanying Figures 1-11, executed by Dr. Timothy Kieffer one of the inventors of the subject application. Exhibit A provides data for another *in vivo* animal model in which a therapeutic protein (leptin) in gut cells was produced in an amount effective to treat obesity and normalize glucose levels.

In brief, a gut K cell line, GTC-1, was genetically modified to produce leptin in response to an inducer, RU486 (paragraphs 8-10). In vitro cell culture studies indicated that the cells expressed leptin which was secreted into the medium in response to RU486 in a time and dose-dependent manner (paragraphs 11-14; see also, Figures 1-9). Transformed GTC-1 cells (GTC-1 pSwitch) were then subsequently implanted into mice along with an RU486 pellet (paragraph 15; see, also, Figures 10 and 11). Following implantation, the mice lost weight, and the weight loss continued for up to two weeks until RU486 depletion (paragraph 16). Analysis of blood glucose levels following transplantation revealed that the secreted leptin normalized blood glucose levels in the animals, even after RU486 depletion (paragraph 17). Thus, the data described in Exhibit A corroborates that contacting transformed mucosal tissue cells in a subject with a nutrient that induces production of a protein in an amount effective to treat the disorder is enabled, as is claimed.

As to levels of therapeutic protein expression for "a long enough period of time," the claimed methods merely require that cells within the gut express the therapeutic protein at levels

effective to treat the disorder. In the case of insulin for example, the specification discloses a variety of clinically relevant criteria that satisfy this endpoint including, for example, *inter alia*, reduced blood glucose, improved glucose tolerance, normal glucose homeostasis, etc. (page 35, lines 2-5). The claims do not require that the therapeutic effect last for a specified amount of time. Thus, the Patent Office's grounds for rejection due to expression of adequate levels for "a long enough period of time" is inapplicable to the present claims as they are not so limited. Furthermore, both the transgenic insulin animal model exemplified in the specification and the implanted leptin cell animal model described in Exhibit A demonstrate that a therapeutic effect is detectable by a variety of clinically relevant criteria including glucose normalization and weight loss. Accordingly, the ground for rejection should properly be withdrawn.

As to cell targeting, transformed cells include mucosal cells such as gut K-cells, as well as gut stem cells, which are progenitor cells of K-cells. In the *in vivo* animal insulin model exemplified in the specification, transforming gut stem cells likely results in sustained insulin production (page 25, lines 24-25). Because stem cells are included as targets, which provide a source of differentiated K-cells, longer term expression is expected, and was achieved in the transgenic insulin animal model exemplified in the specification.

In support of this position, Applicants submit herewith as Exhibit B, a publication by During *et. al.* (Nature Med. 4:1131 (1998)). These authors demonstrated long-term (6 month) *in vivo* expression of β-galactosidase in gut cells with a single dose of orally administered adeno-assiociated virus (AAV). Transgene expression was localized to epithelial cells in the gut mucosa. Since the life span of gut epithelial cells is short (3-5 days), the long-term expression achieved indicates that the transgene is likely incorporated into gut stem cells. In further support of this position, Applicants submit herewith as Exhibit C, a publication by During et. al. (Science 287:1453-58 (2000)). These authors describe data indicating that *in vivo* expression in the intestine persisted for at least 5 months with a single peroral delivery of AAV vectors. Accordingly, given that delivery systems for gut expression were known in the art at the time of the invention, the grounds for rejection relating to gut cell delivery should properly be withdrawn.

As to the Yoon et al. reference cited in the Office Action, the specification exemplifies that insulin was secreted in a nutrient-regulated fashion in gut cells, at levels effective to reduce hyperglycemia in an animal model and, that animals expressing insulin were resistant to

symptoms associated with diabetes following pancreas β-cell destruction. Thus, the specification indicates that non-β cells can be used for insulin production, that such cells secrete insulin in response to glucose, and that the insulin produced is properly processed. Moreover, given the reduction in glucose levels, the insulin produced was obviously biologically active. Thus, given that the concerns mentioned by Yoon et al. have been adequately addressed, one cannot objectively argue that the Yoon et al. reference suggests that claims 31 to 55 lack enablement.

As to the Verma and Crystal references, these references discuss general problems associated with *in vivo* gene therapy. Again however, as discussed above the claims do not require *in vivo* gene therapy. Furthermore, the Verma and Crystal references are irrelevant to *ex vivo* and other methods of introducing transformed cells or genetic material into animals. Moreover, neither of Verma nor Crystal discuss targeting mucosal cells, which as the data in Exhibits B and C discussed above indicate have been transformed and produce relatively sustained protein levels.

Thus, in view of the fact that claims 31 to 55 do not require *in vivo* gene therapy, the exemplified animal model and the animal model described in Exhibit A indicating that therapeutic levels of insulin and leptin can be produced *in vivo* as is claimed, and further in view of Exhibits B and C, claims 31 to 55 are adequately enabled. Accordingly, Applicants respectfully request that the rejection under 35 U.S.C. §112, first paragraph, as allegedly lacking enablement, be withdrawn.

CONCLUSION

In summary, for the reasons set forth herein, Applicants maintain that claims 31 to 55 clearly and patentably define the invention, respectfully request that the Examiner reconsider the various grounds set forth in the Office Action, and respectfully request the allowance of the claims which are now pending.

If the Examiner would like to discuss any of the issues raised in the Office Action, Applicant's representative can be reached at (858) 509-4065.

Please charge any additional fees, or make any credits, to Deposit Account No. 03-3975.

Respectfully submitted,

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